BIOLOGICAL MARKERS AND STABLE CARBON ISOTOPE STUDIES OF BITUMEN IMPREGNATING QUATERNARY SEDIMENTS AT STARUNIA PALAEONTOLOGICAL SITE AND VICINITY (CARPATHIAN REGION, UKRAINE)

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Abstract: Biological markers and stable carbon isotopic compositions of bitumen impregnating the Pleistocene and Holocene sediments around the Starunia palaeontological site (Carpathian region, Ukraine) near discovered remnants of a mammoth and three woolly rhinoceroses, and one almost completely preserved rhinoceros carcass found in 1907 and 1929, were investigated. The research was carried out to assess genetic connections between the bitumen and oils from the nearby deep accumulations of the Boryslav-Pokuttya and Skyba units of the Carpathians. Another aim of these geochemical studies was to reveal the secondary geochemical processes (oxidation and biodegradation) influencing the bitumen, and to determine which environments have been favourable or unfavourable for the preservation of large Pleistocene vertebrates. Bitumen included within the near-surface rocks are not genetically connected with residual organic matter present in Quaternary sediments. Geochemical features, such as distributions of pentacyclic triterpanes and steranes, biomarker parameters and isotope composition indicate that all bitumens originate from oil-prone, Type II kerogen with insignificant admixture of terrestrial Type III kerogen in the middle stage of catagenesis. The oils occurring in deep accumulations in the Starunia area were the sole source of bitumen found in the near-surface sediments. Input of immature organic matter to bitumen from Pleistocene and Holocene sediments has not been found. The main factors differentiating the bitumen were: biodegradation, water washing and/or weathering. Additional influence of transport of bitumen by brine was found. The most favourable conditions for preservation of large, extinct mammals within the Pleistocene muds exist in the vicinity of Nos 22 and 23 boreholes, where bitumen is best preserved. The worst conditions were found in the vicinity of Nos 1, 4, 4' and 15 boreholes. Intensification of biodegradation and weathering effects were followed by intensive, chaotic changes of the remnants of large Pleistocene mammals.

Key words: biomarkers, stable carbon isotopes, bitumen content, oil origin, woolly rhinoceros, Starunia palaeontological site, Carpathian region, Ukraine.

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INTRODUCTION

Geochemical studies including fraction analysis, biomarker distribution and stable carbon isotopes of bitumen that saturate Pleistocene and Holocene sediments were an important part of an interdisciplinary research project focused on study of the Starunia area. In the years 2006–2009, this project was run in the area of the Starunia abandoned ozokerite (earth wax) mine (Kotarba, 2009), about 130 kilometres southeast of Lviv, Ukraine (Fig. 1). At the end of the 19th century and in first half of the 20th century, ozokerite (earth wax) was exploited in the Starunia area from the

Boryslav-Pokuttya Unit of the Carpathian Foredeep Basin (Alexandrowicz, 2004, 2005; Koltun *et al.*, 2005).

Starunia has been famous since 1907 as the site, where remains of woolly rhinoceros and mammoth were found at depths 12–17 m below the surface at the place where ozokerite was mined. This exceptional discovery was followed by one in 1929 when almost completely preserved carcass of woolly rhinoceros was excavated in the special shaft dug by the Polish Academy of Arts and Sciences in Quaternary muds saturated with oil and brine (Kotarba, 2002; Alexan-
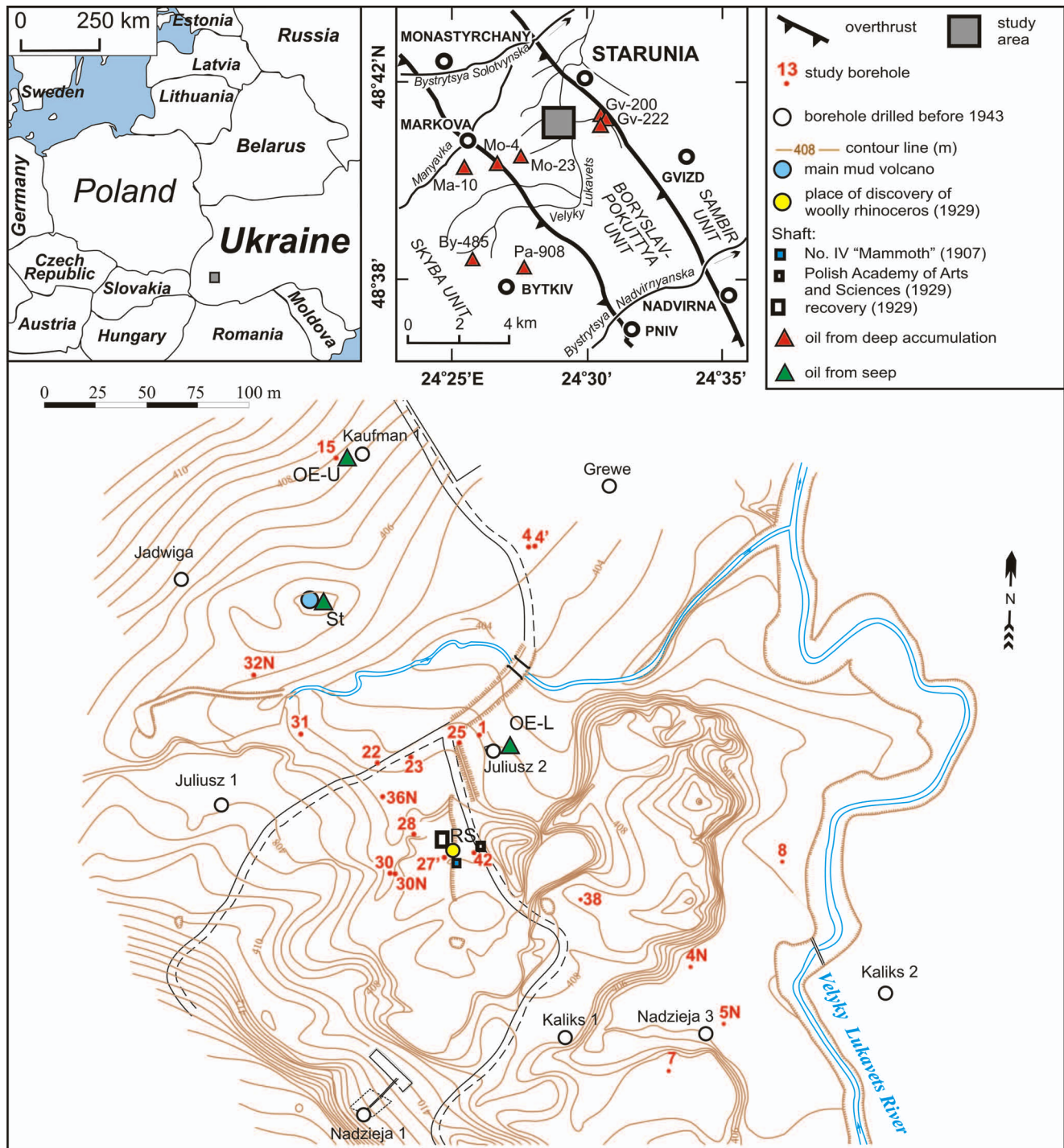
Fig. 1. Sketch map of the Starunia palaeontological site and vicinity (Carpathian region, Ukraine) showing the location of boreholes, sampling sites of oil derived from deep accumulations and surface seeps, and position of oil sample taken from skull of woolly rhinoceros, after Kotarba *et al.* (2005b): OE-U – oil from "upper oil eye", OE-L – oil from "lower oil eye", St – oil from mud volcano, RS – oil collected in 1907 directly from skull of the "second" woolly rhinoceros by an unknown Polish geologist (probably M. Łomnicki)

drowicz, 2005). The discovery of large Pleistocene mammals in the Starunia ozokerite mine was a spectacular scientific event on a global scale. An unique combination of oil and brine saturating the Pleistocene muds, into which the animals had sunk, resulted in nearly perfect preservation of these specimens. Both salt (chloride ion) and bitumen (oil) were deciding factors in impregnation, conservation and

preservation of large, extinct mammals embedded within the Pleistocene sediments (Kotarba, 2002; Kotarba *et al.*, 2005b, 2008b, 2009b; Mościcki *et al.*, 2009).

The main aim of this geochemical study covering methods of bitumen saturating Pleistocene and Holocene sediments was to determine the genetic connections between these bitumens and oils from deep accumulations in both the

Boryslav-Pokuttya and Skyba units, from surface seeps, and from a single oil sample collected in 1907 directly from the skull of woolly rhinoceros. Moreover, the influence was revealed of secondary geochemical processes (oxidation and biodegradation) active within these sediments, especially in Pleistocene muds from the Starunia area. Therefore, we paid special attention to the occurrence of such processes in the studied strata. We expect that solution of this problem will enable us to determine which environments have been favourable or unfavourable for the preservation of large, Pleistocene vertebrates.

General information covering the history of the Starunia area and the results of earlier research, including organic geochemical study and genetic correlations between source rocks and hydrocarbons from surface seeps and deep accumulations carried out in the area (Kotarba *et al.*, 2005b), are presented in a special monograph devoted to Starunia (Kotarba, *ed.*, 2005).

Organic matter contained in Pleistocene and Holocene sediments is of both syngenetic (*e.g.* molluscs and plant macrofossils) (Stachowicz-Rybka *et al.*, 2009) and epigenetic (oil migrating from deep structures of the Boryslav-Pokuttya and Skyba units to Quaternary sediments) origins (Kotarba, 2002; Kotarba *et al.*, 2005b, 2009b; Koltun *et al.*, 2005). Inflow of oil and thermogenic gases from such deep accumulations to Quaternary sediments and development of microbial and secondary geochemical processes within these sediments in the Starunia area were revealed by Kotarba *et al.* (2005a, 2009a), Sechman *et al.* (2009), and Barabasz *et al.* (2009).

GEOLOGICAL SETTING
AND PETROLEUM OCCURRENCE

The Ukrainian Carpathians belong to the largest petroleum provinces of Central Europe, constituting one of the oldest petroleum-producing regions in the world. Exploitation of oil and natural gas began in 1854 and 1921, respectively (Kotarba & Koltun, 2006). Ozokerite is a rare petroleum substance; in the Carpathians it is known from only five sites: Boryslav, Volanka, Truskavets-Pomiarki, Dzvinyach and Starunia (Alexandrowicz, 2005).

In the area of the abandoned Starunia ozokerite mine, Quaternary sediments of the Velyky Lukavets River valley are developed as clayey muds with plant remains, peat, biogenic muds, and peat muds (Sokołowski *et al.*, 2009; Sokołowski & Stachowicz-Rybka, 2009). Details of geology and petroleum occurrence in the Starunia area were published by Alexandrowicz (2004, 2005), Koltun *et al.* (2005), Korin (2005), Kotarba & Stachowicz-Rybka (2008), Kotarba *et al.* (2008a), Sokołowski *et al.* (2009), Sokołowicz & Stachowicz-Rybka (2009), Stachowicz-Rybka *et al.* (2009), and in references therein.

The top surface of the salt-bearing Miocene Vorotyshcha beds in Starunia area, which underlie Quaternary deposits, occurs at a maximum depth of 17 metres (Sokołowski *et al.*, 2009). The Miocene strata are sandstone-claystone breccias with halite, potassium-salt, gypsum and calcite layers, and veins of ozokerite (Korin, 2005). Within Quaternary

sediments, many brine and salt water springs occur in the vicinity of Starunia. These waters are genetically connected with dissolution of salts and leakage from the Miocene Vorotyshcha beds (Duliński *et al.*, 2005). The Boryslav-Pokuttya Unit is the main oil and gas reservoir in the Ukrainian Carpathians. Tectonically, the unit represents a stack of superimposed nappes, each of them comprising the flysch sequence covered by molasses. The Oligocene Menilite beds occur in the top of the flysch succession and are considered to be the most important hydrocarbon source rock with relatively high organic matter content (up to 20 wt%; Kotarba & Koltun, 2006). South and southwest of the Starunia ozokerite deposit, six oil and gas fields were discovered within the Palaeogene and Neogene reservoirs of the Boryslav-Pokuttya and Skyba units (Adamenko *et al.*, 2005; Koltun *et al.*, 2005). The salt-bearing Miocene Vorotyshcha beds provide perfect sealing of hydrocarbon traps in the Boryslav-Pokuttya Unit. However, these beds underwent considerable cracking in the course of formation of the Starunia Fold, during the Carpathian overthrust movements. As oil and gas were flowing from the flysch strata towards the surface and most of gaseous hydrocarbons were emitted to the atmosphere, liquid hydrocarbons saturated Quaternary sediments, and higher hydrocarbons formed veins of ozokerite. These processes continue even now, which can be seen on the example of oil and gas surface seeps and mud volcanoes in the Starunia area (Kotarba, 2009).

METHODS

Sampling procedure

For geochemical studies 113 core samples were taken (Kotarba *et al.*, 2009b). For biological marker and stable carbon isotope analyses 56 core samples were selected, of which 37 were from Pleistocene sediments and 14 from Holocene strata. For comparison, 4 samples of salt-bearing Miocene Vorotyshcha beds and 1 sample of mine wastes (dump) were analysed, as well. Location of sampled wells is shown in Fig. 1.

Analytical methods

The procedure of bitumen extraction and separation of fractions (saturated and aromatic hydrocarbons and resins) was published by Kotarba *et al.* (2009b). The extracts were analysed with the gas chromatography/mass spectrometry technique using the Agilent Technologies 7890A gas chromatograph with DB-5MS capillary column (60 m x 0.32 mm internal diameter, 0.25 µm stationary phase), directly coupled to the 5975C Network mass detector. Injector of "Cool On Column" type was used. Sample volume was 0.5 µl. The following measurement parameters were applied: carrier gas – He; programmed temperature: 313 K (isothermal for 1 min.), heating rate to 393 K – 20 K/min, then to 573 K – 3 K/min, final isothermal temperature: 573 K hold for 35 min. The mass-selective detector was operating in the electron impact ionization mode at 70 eV and scanned from 45 to 500 Da. Data were acquired in a full-
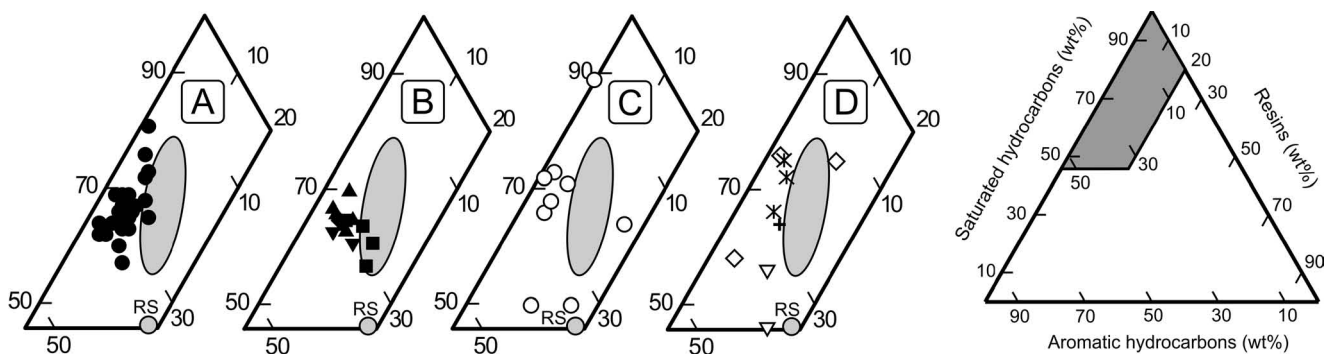
Fig. 2. Ternary diagram of saturated hydrocarbons, aromatic hydrocarbons and resins from bitumen extracted from: (**A**) Pleistocene clayey muds, (**B**) Pleistocene peat, biogenic muds and muds saturated with bitumen, (**C**) Holocene clayey muds, and (**D**) Holocene peat muds and muds saturated with bitumen, salt-bearing Miocene Vorotyshcha beds, and mine wastes. Shaded area represents oils from surface seeps, from skull of the "second" woolly rhinoceros (RS) in Starunia, and the nearest deep accumulations, after Kotarba *et al.* (2005b). For explanation of symbols see Fig. 4

scan mode and processed with the Hewlett Packard – Agilent Technologies Chemstation software. All compounds were identified using their mass spectra, comparison of retention times of their peaks to standard compounds and literature data, and interpretation of MS fragmentation patterns (Philp, 1985; Peters *et al.*, 2005; MSD, 2000). The objective of gas chromatography/mass spectrometry analysis was to recognize the distribution of characteristic biomarkers using peak areas found in the suitable ion chromatograms.

Rock samples selected for stable carbon isotope analysis of insoluble organic matter were combusted in an on-line system after removal of carbonates with hydrochloric acid and extraction of bitumen. Previously extracted bitumen and its fractions for stable carbon isotope analyses were carried on with the same procedure. Stable carbon isotope analyses were run with the Finnigan Delta Plus mass spectrometer. Stable carbon isotope data were presented in δ-notation relative to the PDB standard. Analytical precision was estimated as ± 0.2‰.

RESULTS AND DISCUSSION

Bitumen composition

The fraction composition of bitumen extracted from the near-surface sediments in the study area is presented in Table 1. Bitumen is rich in saturated hydrocarbons (from 40 to 89 wt%, median 65 wt%) and aromatic hydrocarbons (from 11 to 40 wt%, median 29 wt%); resin contents were low, from 0 to 17 wt% (median 5 wt%) (Table 1, Fig. 2). Bitumen extracted from the Pleistocene clayey muds (Fig. 2A) is richest in hydrocarbons, whereas the highest resin contents are observed in the Holocene clayey muds (Fig. 2C). Fraction composition of analysed bitumen corresponds to that of oils accumulated in the Starunia area (Kotarba *et al.*, 2005b) and to bitumen extracted from rhinoceros bones (Kotarba, 2002). Only the oil collected from rhinoceros skull in 1907 was richer in resins (RS sample in Fig. 2A). These differences probably result from long-lasting sample storage as

well as from evaporation and biodegradation processes, which reduced the content of saturated hydrocarbons (Peters *et al.*, 2005).

Biomarker analysis

n-Hexane extracts (bitumen) from the near-surface Pleistocene and Holocene sediments in the Starunia area contain *n*-alkanes, acyclic isoprenoids (mainly pristane and phytane), pentacyclic triterpanes and steranes. These compounds were used for calculations of geochemical parameters presented in Table 2.

In most samples the saturated hydrocarbon fraction of bitumen is dominated by *n*-alkanes, except for samples Nos 4/3.5, 4/4.0, 4'/3.7, 4'/4.85 30/4.6, 30/5.8 and 30/6.8, which do not contain these compounds and, instead, pristane (Pr) and phytane (Ph) display the highest concentrations. Examples of *n*-alkanes and acyclic isoprenoids distributions (*m/z* = 71) of saturated hydrocarbon fractions are presented in Fig. 3. Both the distribution pattern and the range of compounds are variable. *n*-Alkane distribution is usually monomodal. Long-chain (n-C_{23} – n-C_{35}) or short-chain (n-C_{13} – n-C_{22}) *n*-alkanes may prevail in the extracts, as shown by Σ1/Σ2 ratios of these groups (Table 2).

There is a reverse trend between chloride ion content and Σ1/Σ2 values (Fig. 4). For samples containing over 2 wt% of chloride ions long-chain *n*-alkanes usually dominate whereas short-chain hydrocarbons prevail mainly in low-chloride samples, regardless sediment age and lithology (Fig. 4).

n-Alkane distribution shows a smooth outline in most of the extracts, with the Carbon Preference Index (CPI) values varying from 1.00 to 2.03 and with most of values clustering around 1.17 (Table 2). The CPI values, which express predominance of odd-over-even numbered carbon atoms *n*-alkanes, decrease to about 1.00 with the increasing thermal maturity, suggesting some input of terrestrial organic matter (Tissot & Welte, 1984). Comparing these results with other maturity parameters shown in Table 2 and considering the absence of unsaturated compounds (which is typical of immature organic matter), we propose that thermally mature bitumen and/or oil migrating from deep accu-

Table 1

Fractions and stable carbon isotope composition of bitumens, their individual fractions and insoluble organic matter from Starunia area

Sample well/depth (m)	Strati-graphy	Lithology	Fractions (wt%)			Stable carbon isotope composition (‰)				
			Sat.	Aro.	Res.	Sat.	Bit.	Aro.	Res.	I.O.M.
1/2.6	Ple.	Biogenic mud	63	30	7	-26.2	-26.1	-25.8	-26.7	n.a.
4/3.5	Ple.	Clayey mud	66	30	4	-26.2	-25.9	-25.6	-26.0	n.a.
4/4.0	Ple.	Clayey mud	60	33	7	-26.1	-25.8	-25.6	-26.0	n.a.
4/5.0	M.	V.s-b.b.	66	28	6	-26.1	-25.8	-25.6	-26.0	-23.3
4'/1.0	Hol.	Clayey mud	50	34	16	n.a.	n.a.	n.a.	n.a.	n.a.
4'/3.7	Ple.	Biogenic mud	67	30	3	-26.1	-26.0	-25.8	-26.5	n.a.
4'/4.0	Ple.	Clayey mud	68	25	7	-26.1	-26.1	-25.8	-26.7	n.a.
4'/4.65	Ple.	Biogenic mud	66	30	4	-26.1	-26.1	-25.7	-26.3	n.a.
4'/4.85	Ple.	Clayey mud	62	35	3	-26.3	-25.9	-25.8	-26.0	-27.1
4N/4.15	Hol.	Clayey mud	73	25	2	-26.3	-26.3	-25.7	-27.0	-26.7
5N/6.5	Hol.	Clayey mud	72	27	1	-26.4	-26.3	-25.9	-27.0	n.a.
7/10.4	Hol.	Clayey mud	71	24	5	-26.5	-26.2	-25.9	-27.8	n.a.
8/1.5	Hol.	Clayey mud	89	11	0	-26.6	-26.5	-26.4	-26.6	n.a.
15/0.2	Ple.	Clayey mud	81	18	1	-26.6	-26.5	-26.0	-26.9	n.a.
15/2.0	Ple.	Clayey mud	57	34	9	-26.8	-26.4	-25.7	-27.0	n.a.
15/4.0	Ple.	Clayey mud	63	31	6	-26.3	-26.2	-25.8	-26.9	n.a.
15/6.0	Ple.	Clayey mud	63	30	7	-26.3	-26.2	-25.9	-26.8	n.a.
22/1.0	Hol.	Mud sat.	56	34	10	-26.6	-26.4	-26.3	-26.9	-26.7
22/2.5	Ple.	Clayey mud	72	23	5	-26.6	-26.5	-26.1	-27.2	-28.1
22/3.55	Ple.	Peat	61	27	12	-27.4	-27.4	-26.4	-28.8	-29.5
22/4.0	Ple.	Clayey mud	76	21	3	n.a.	n.a.	n.a.	n.a.	n.a.
22/4.8	Ple.	Peat	64	27	9	-27.0	-26.9	-26.1	-27.5	-28.8
22/5.5	Ple.	Peat	57	30	13	-26.6	-26.7	-26.0	-27.4	-28.8
22/6.6	M.	V.s-b.b.	75	22	3	-27.0	-26.8	-26.3	-27.1	n.a.
22/7.9	M.	V.s-b.b.	72	23	5	-26.9	-26.7	-26.2	-26.8	n.a.
23/2.0	Ple.	Clayey mud	66	28	6	-26.3	-26.2	-25.9	-27.1	n.a.
23/4.0	Ple.	Clayey mud	67	27	6	-26.6	-26.5	-26.1	-28.0	-28.4
25/1.7	Hol.	Peat mud	58	38	4	-26.3	-26.1	-25.6	-26.5	-26.3

Sample well/depth (m)	Strati-graphy	Lithology	Fractions (wt%)			Stable carbon isotope composition (‰)				
			Sat.	Aro.	Res.	Sat.	Bit.	Aro.	Res.	I.O.M.
25/2.6	Ple.	Biogenic mud	64	30	6	-26.5	-26.2	-25.9	-26.9	n.a.
27/9.5	mine dump	Mine dump	64	28	8	-26.6	-26.3	-25.9	-26.5	n.a.
28/3.4	Ple.	Clayey mud	62	34	4	-26.8	-26.5	-26.1	-27.1	n.a.
28/4.7	Ple.	Clayey mud	69	28	3	-26.4	-26.1	-25.8	-26.4	-26.8
28/6.0	Ple.	Clayey mud	64	32	4	-26.4	-26.3	-25.9	-26.4	n.a.
28/9.8	M.	V.s-b.b.	75	22	3	-27.0	-26.7	-26.0	-27.3	-24.7
30/1.4	Hol.	Peat mud	75	14	11	-27.3	-27.1	-26.0	-26.9	-26.4
30/2.05	Hol.	Mud sat.	46	39	15	-26.9	-26.5	-26.0	-26.3	-26.4
30/4.6	Ple.	Clayey mud	64	34	2	-26.4	-26.3	-26.0	-26.2	n.a.
30/5.8	Ple.	Clayey mud	67	29	4	-26.4	-26.3	-26.0	-26.3	n.a.
30/6.8	Ple.	Peat mud	65	30	5	-26.5	-26.4	-26.1	-26.3	n.a.
30/7.2	Ple.	Mud sat.	61	30	9	-26.7	-26.6	-26.1	-26.9	n.a.
30N/2.9	Ple.	Clayey mud	73	22	5	n.a.	n.a.	n.a.	n.a.	n.a.
30N/4.6	Ple.	Biogenic mud	70	26	4	n.a.	n.a.	n.a.	n.a.	n.a.
30N/5.0	Ple.	Biogenic mud	65	28	7	-26.6	-26.3	-26.1	-26.5	-26.0
30N/7.2	Ple.	Clayey mud	63	31	6	n.a.	n.a.	n.a.	n.a.	n.a.
30N/7.3	Ple.	Clayey mud	64	31	5	-26.4	-26.3	-26.4	-26.5	n.a.
30N/8.2	Ple.	Peat	65	29	6	-26.4	-26.5	-26.0	-27.9	-26.0
30N/8.8	Ple.	Clayey mud	65	29	6	-26.5	-26.5	-26.1	-27.5	n.a.
31/4.0	Hol.	Clayey mud	66	30	4	n.a.	n.a.	n.a.	n.a.	n.a.
32N/2.0	Hol.	Clayey mud	50	40	10	-27.1	-26.7	-26.2	-28.0	n.a.
32N/5.0	Hol.	Peat mud	76	22	2	-26.6	-26.5	-26.2	-27.3	n.a.
38/12.90	Ple.	Clayey mud	69	29	2	-26.3	-26.2	-25.9	-26.8	n.a.
42/3.8	Hol.	Clayey mud	64	19	17	-26.9	-26.7	-26.3	-26.7	n.a.
42/4.6	Hol.	Clayey mud	68	28	4	-26.4	-26.3	-25.9	-26.2	n.a.
42/6.45	Ple.	Clayey mud	69	27	4	-26.4	-26.3	-25.8	-27.1	n.a.
42/8.4	Ple.	Mud sat.	63	32	5	-26.5	-26.2	-26.0	-26.6	n.a.
42/8.5	Ple.	Clayey mud	65	26	9	-26.4	-26.3	-25.9	-26.8	-26.1

Ple. – Pleistocene; Hol. – Holocene; M. – Miocene; Sat. – Saturated hydrocarbons; Aro. – Aromatic hydrocarbons; Res. – Resins; I.O.M. – Insoluble organic matter; n.a. – not analysed

V.s-b.b. – Vorotyshcha salt-bearing beds; mud sat. – mud saturated by bitumen

Biomarker indices of bitumen and chloride ion content from Starunia area

Table 2

Sample, well/depth (m)	CPI	$C_{31}\alpha\beta$22S/ (22S+22R)	Ts/ (Tm+Ts)	$C_{30}\beta\alpha$/ ($\alpha\beta+\beta\alpha$)	29Ts/ (C_{29}+29Ts)	OI/C_{30} hop	$\Sigma1/\Sigma2$	16/17	22/23	13–18 (%)	19–24 (%)	25–40 (%)	Pr/Ph	Pr/ n-C_{17}	Ph/ n-C_{18}	C_{29} 20S/ (S+R)	$C_{29}\alpha\beta\beta$/ ($\alpha\alpha\alpha+\alpha\beta\beta$)	C_{29}/C_{27} ster	C_{27} dia/ C_{27} reg	Pr/C_{30} hop	Cl⁻ content (wt%)
1/2.6	0.61	0.55	0.37	0.21	0.15	0.18	0.60	76.1	0.91	14	56	30	1.47	26.7	0.19	0.26	0.30	1.22	0.36	0.07	0.00
4/3.5	-	0.56	0.41	0.16	0.17	0.20	-	-	-	-	-	-	-	-	-	0.36	0.36	1.10	0.40	-	0.00
4/4.0	-	0.56	0.42	0.21	0.16	0.18	-	-	-	-	-	-	-	-	-	0.35	0.36	0.99	0.39	0.08	0.00
4/5.0	-	0.56	0.43	0.21	0.17	0.18	0.25	0.00	1.38	18	71	11	1.53	12.5	7.1	0.34	0.34	1.04	0.36	1.13	0.00
4'/1.0	1.77	0.57	0.30	0.17	0.23	0.41	1.55	6.7	1.62	21	24	54	1.58	12.5	1.06	0.41	0.39	1.77	0.19	0.40	0.00
4'/3.7	-	0.56	0.40	0.20	0.17	0.17	-	-	-	-	-	-	1.78	-	-	0.38	0.38	1.04	0.38	2.32	0.00
4'/4.0	1.83	0.55	0.48	0.21	0.19	0.19	1.88	3.62	0.32	7	32	61	1.49	49.6	9.6	0.32	0.35	1.30	0.35	0.84	0.00
4'/4.65	2.00	0.56	0.41	0.18	0.17	0.18	0.52	5.4	1.84	13	57	30	1.70	58.1	7.4	0.34	0.35	0.91	0.30	0.29	0.00
4'/4.85	-	0.55	0.41	0.17	0.18	0.17	-	-	-	-	-	-	1.82	-	-	0.35	0.35	1.05	0.46	0.42	0.00
4N/4.15	1.22	0.54	0.42	0.18	0.20	0.19	3.86	9.8	1.01	6	22	71	2.08	2.55	0.09	0.35	0.37	1.33	0.27	0.06	0.00
5N/6.5	2.03	0.57	0.38	0.18	0.18	0.19	1.01	11.8	2.20	21	33	46	1.32	20.3	1.92	0.34	0.36	0.99	0.27	0.54	1.18
7/10.4	1.81	0.56	0.44	0.18	0.18	0.18	0.62	0.66	1.87	18	49	33	1.66	14.0	12.8	0.35	0.36	0.99	0.29	2.31	0.00
8/1.5	1.05	0.57	0.41	0.16	0.19	0.16	3.89	0.84	0.83	5	20	75	1.79	1.28	0.54	0.34	0.35	0.90	0.33	4.02	0.18
15/0.2	1.08	0.57	0.41	0.21	0.17	0.19	38.0	4.75	1.39	1	2	97	1.75	15.8	2.10	0.38	0.34	1.10	0.26	0.98	0.33
15/2.0	1.05	0.57	0.31	0.22	0.38	0.31	2.54	0.92	1.09	7	27	66	1.44	3.63	0.86	0.53	0.43	1.05	0.13	0.30	0.51
15/4.0	2.02	0.56	0.35	0.21	0.16	0.19	0.77	2.30	1.71	10	54	37	1.44	37.4	6.1	0.34	0.35	0.93	0.29	0.77	0.65
15/6.0	1.70	0.55	0.44	0.21	0.18	0.18	1.55	2.45	1.98	17	29	53	1.68	14.1	4.10	0.36	0.38	1.17	0.43	0.37	0.43
22/1.0	1.00	0.56	0.44	0.22	0.18	0.22	1.88	8.9	1.00	12	29	59	1.10	2.89	0.18	0.33	0.34	1.37	0.30	0.07	4.17
22/2.5	1.19	0.55	0.35	0.20	0.15	0.20	0.26	0.60	1.19	25	61	13	1.58	1.40	0.71	0.34	0.34	1.03	0.35	3.86	2.46
22/3.55	1.03	0.56	0.38	0.22	0.14	0.16	0.36	3.96	1.93	38	43	20	1.63	3.82	1.22	0.42	0.38	0.92	0.21	3.16	4.66
22/4.0	1.32	0.55	0.34	0.18	0.16	0.18	0.54	0.54	1.07	15	59	26	1.42	1.38	0.59	0.29	0.31	1.24	0.38	1.62	2.80
22/4.8	0.87	0.57	0.38	0.22	0.16	0.23	0.28	2.32	1.76	40	45	16	1.92	1.88	0.84	0.40	0.36	1.05	0.12	1.68	3.24
22/5.5	1.32	0.57	0.40	0.21	0.16	0.20	0.26	0.76	1.27	19	60	22	1.44	2.11	0.69	0.38	0.36	1.44	0.34	0.99	4.61
22/6.6	1.05	0.55	0.37	0.20	0.16	0.19	0.91	1.20	0.88	19	44	37	1.68	1.10	0.26	0.34	0.36	0.94	0.41	4.83	3.02
22/7.9	1.03	0.55	0.36	0.19	0.17	0.16	0.76	1.38	1.01	15	51	33	1.53	1.26	0.33	0.31	0.33	1.14	0.35	3.99	7.13
23/2.0	1.18	0.56	0.41	0.17	0.16	0.21	0.67	1.08	1.49	23	45	33	0.29	0.19	0.90	0.32	0.36	1.01	0.28	0.10	0.34
23/4.0	1.25	0.56	0.38	0.22	0.16	0.21	0.59	2.60	1.18	15	56	29	2.00	60.5	9.1	0.32	0.33	0.98	0.29	3.66	0.99
25/1.7	1.37	0.55	0.34	0.18	0.16	0.19	0.67	8.3	2.86	15	53	31	1.21	11.4	9.1	0.30	0.33	1.23	0.37	0.13	0.00
25/2.6	1.03	0.57	0.38	0.21	0.15	0.18	0.60	5.1	3.25	22	48	30	1.47	11.7	8.3	0.34	0.36	1.19	0.36	2.51	0.00
27/9.5	1.09	0.57	0.37	0.18	0.27	0.25	3.07	1.19	0.76	5	27	68	1.96	7.4	1.84	0.39	0.39	0.93	0.20	2.56	1.43
28/3.4	0.51	0.55	0.38	0.20	0.15	0.19	0.29	7.3	5.1	45	39	16	1.61	12.0	2.96	0.31	0.33	1.07	0.33	2.30	0.70
28/4.7	2.48	0.55	0.48	0.21	0.19	0.18	0.21	1.09	1.24	29	59	11	1.86	16.3	10.2	0.35	0.33	1.15	0.34	2.62	0.86
28/6.0	1.77	0.55	0.40	0.21	0.16	0.17	0.15	1.14	1.33	39	55	7	1.72	9.1	6.9	0.34	0.30	0.97	0.36	2.74	0.94
28/9.8	1.00	0.56	0.34	0.20	0.16	0.22	0.31	1.27	1.21	34	50	16	1.76	1.28	0.70	0.38	0.36	1.11	0.34	4.22	3.46
30/1.4	0.60	0.58	0.30	0.23	0.45	0.58	0.39	33.3	5.1	49	29	23	1.38	4.10	0.35	0.60	0.46	3.47	0.67	0.13	0.68
30/2.05	0.90	0.56	0.38	0.21	0.35	0.36	0.25	2.15	1.08	36	48	16	2.18	5.3	3.07	0.54	0.46	4.41	0.89	0.02	0.52
30/4.6	-	0.56	0.39	0.18	0.17	0.18	-	-	-	-	-	-	-	-	-	0.31	0.33	1.10	0.38	-	0.16
30/5.8	-	0.56	0.38	0.17	0.17	0.17	-	-	-	-	-	-	1.57	-	-	0.37	0.36	1.19	0.41	1.49	0.00
30/6.8	-	0.56	0.34	0.21	0.17	0.17	-	-	-	-	-	-	2.18	-	-	0.37	0.36	1.08	0.34	5.15	0.18
30/7.2	0.53	0.55	0.38	0.20	0.17	0.19	0.28	5.2	2.38	43	42	15	1.90	13.0	3.37	0.34	0.34	1.05	0.40	4.00	0.59
30N/2.9	1.00	0.56	0.39	0.23	0.21	0.27	1.24	7.4	1.46	17	36	47	1.81	68.5	4.87	0.39	0.40	1.10	0.16	2.14	0.42
30N/4.6	1.01	0.56	0.36	0.22	0.16	0.23	10.2	6.9	1.64	3	8	89	1.56	16.7	1.26	0.43	0.38	1.21	0.18	0.51	0.68
30N/5.0	1.02	0.55	0.36	0.21	0.17	0.23	1.00	19.1	6.56	29	30	41	1.38	34.4	1.30	0.31	0.36	1.48	0.29	0.39	0.68

Table 2 continued

Sample, well/depth (m)	CPI	C31αβ22S/ (22S+22R)	Ts/ (Tm+Ts)	C30βα/ (αβ+βα)	29Ts/ (C29+29Ts)	OI/C30 hop	n-Alkanes Σ1/Σ2	16/17	22/23	13-18 (%)	19-24 (%)	25-40 (%)	Pr/Ph	Pr/ n-C17	Ph/ n-C18	C29 20S/ (S+R)	C29αββ/ (αααα+αββ)	C29/C27 ster	C27 dia/ C27 reg	Pr/C30 hop	Cl- content (wt%)
30N/7.2	0.92	0.55	0.35	0.21	0.19	0.20	0.92	6.1	2.71	29	26	45	1.90	13.0	1.27	0.30	0.33	1.66	0.33	0.79	0.51
30N/7.3	1.01	0.55	0.35	0.21	0.16	0.19	1.79	6.9	2.09	18	21	60	1.76	27.3	2.44	0.30	0.34	1.22	0.32	0.45	0.69
30N/8.2	1.10	0.56	0.37	0.18	0.16	0.19	0.23	1.67	2.02	16	69	15	1.80	34.8	15.3	0.32	0.33	1.02	0.29	2.77	0.00
30N/8.8	1.09	0.56	0.38	0.18	0.16	0.17	1.26	5.9	1.61	10	39	50	1.76	105	12.5	0.33	0.33	0.97	0.28	2.10	0.68
31/4.0	1.50	0.55	0.33	0.20	0.15	0.21	0.38	0.65	1.15	20	60	19	1.68	2.15	0.92	0.34	0.34	1.10	0.34	2.16	1.94
32N/2.0	1.24	0.56	0.41	0.19	0.28	0.08	1.54	15.3	1.91	24	27	49	1.40	3.09	0.13	0.39	0.38	1.24	0.13	0.05	1.31
32N/5.0	1.21	0.57	0.38	0.19	0.15	0.21	0.37	1.56	1.90	21	57	21	1.83	26.3	10.8	0.35	0.34	0.91	0.31	4.13	2.57
38/12.90	1.18	0.56	0.36	0.19	0.18	0.20	0.34	0.79	1.06	26	57	18	1.90	1.82	0.81	0.35	0.35	0.94	0.33	3.82	0.00
42/3.8	1.10	0.55	0.29	0.27	0.30	0.70	16.4	0.00	0.77	1	9	90	1.24	-	0.28	0.66	0.31	0.98	-	0.04	0.76
42/4.6	1.09	0.55	0.38	0.22	0.16	0.23	5.3	5.2	0.80	4	18	78	1.76	7.8	0.88	0.41	0.37	1.46	0.16	0.16	0.77
42/6.45	1.13	0.55	0.37	0.21	0.16	0.21	1.26	27.7	7.1	18	32	50	1.60	122	1.92	0.38	0.36	1.06	0.25	0.82	0.70
42/8.4	1.45	0.56	0.40	0.21	0.15	0.22	0.20	2.11	2.24	31	58	10	2.03	35.5	13.8	0.37	0.38	1.06	0.38	4.27	0.00
42/8.5	1.09	0.56	0.40	0.18	0.17	0.19	0.48	12.6	1.10	12	62	26	1.89	277	12.2	0.32	0.35	1.09	0.35	3.07	0.17

CPI (Carbon Preference Index) = $0.5\{[(n\text{-}C_{25}+n\text{-}C_{27}+n\text{-}C_{29}+n\text{-}C_{31}+n\text{-}C_{33})/(n\text{-}C_{24}+n\text{-}C_{26}+n\text{-}C_{28}+n\text{-}C_{30}+n\text{-}C_{32})]+$
$+[(n\text{-}C_{25}+n\text{-}C_{27}+n\text{-}C_{29}+n\text{-}C_{31}+n\text{-}C_{33})/(n\text{-}C_{26}+n\text{-}C_{28}+n\text{-}C_{30}+n\text{-}C_{32}+n\text{-}C_{34})]\}$; (Bray & Evans, 1963);

$C_{31}\alpha\beta$ 22S/(22S+22R) = a ratio of C_{31}-17α,21β(H)-29-homohopane 22S to a sum of its diastereomers 22S and 22R (Ensminger et al., 1977);

Ts/(Tm+Ts) = 18α(H)-22,29,30-trisnorneohopane/(17α(H)-22,29,30-trisnorhopane + 18α(H)-22,29,30-trisnorneohopane) (Seifert & Moldowan, 1978);

$C_{30}\beta\alpha/(\alpha\beta+\beta\alpha)$ = C_{30}-17β,21α (H)-29-hopane/(C_{30}-17α,21β(H)-29-hopane + C_{30}-17β,21α (H)-29-hopane);

29Ts/(C_{29}+29Ts) = 18α-30-norneohopane/(18α-30-norneohopane + C_{30}-17α,21β(H)-29-hopane); OI/C_{30} hop = oleananes/C_{30}-17β,21α (H)-29-hopane;

Σ1/Σ2 = (from $n\text{-}C_{13}$ to $n\text{-}C_{22}$)/(from $n\text{-}C_{23}$ to $n\text{-}C_{35}$); 16/17 = $n\text{-}C_{16}/n\text{-}C_{17}$; 22/23 = $n\text{-}C_{22}/n\text{-}C_{23}$; 13-18 = (Σ from $n\text{-}C_{13}$ to $n\text{-}C_{18}$)/(Σ from $n\text{-}C_{13}$ to $n\text{-}C_{40}$);

19-24 = (Σ from $n\text{-}C_{19}$ to $n\text{-}C_{24}$)/(Σ from $n\text{-}C_{13}$ to $n\text{-}C_{40}$); 25-40 = (Σ from $n\text{-}C_{25}$ to $n\text{-}C_{40}$)/(Σ from $n\text{-}C_{13}$ to $n\text{-}C_{40}$);

Pr/Ph = pristane to phytane ratio (Didyk et al., 1978); Pr/$n\text{-}C_{17}$ = pristane/n-heptadecane (Tissot et al., 1971); Ph/$n\text{-}C_{18}$ = phytane/n-octadecane (Tissot et al., 1971);

C_{29} 20S/(S+R) = a ratio of C_{29}-5α,14α,17α(H)-stigmastane 20S to a sum of its diastereomers 20S and 20R (Seifert & Moldowan, 1986);

$C_{29}\alpha\beta\beta/(\alpha\alpha\alpha+\alpha\beta\beta)$ = a ratio of C_{29}-5α,14β,17β(H)-stigmastane (20S + 20R) to a sum of its distereomers C_{29}-5α,14α,17α(H)-stigmastane (20S+20R) +
C_{29}-5α,14β,17β(H)-stigmastane (20S + 20R) (Seifert & Moldowan, 1986);

C_{29}/C_{27} ster = a ratio of a sum of regular steranes C_{29} to a sum of regular steranes C_{27} (Huang & Meinshein, 1979);
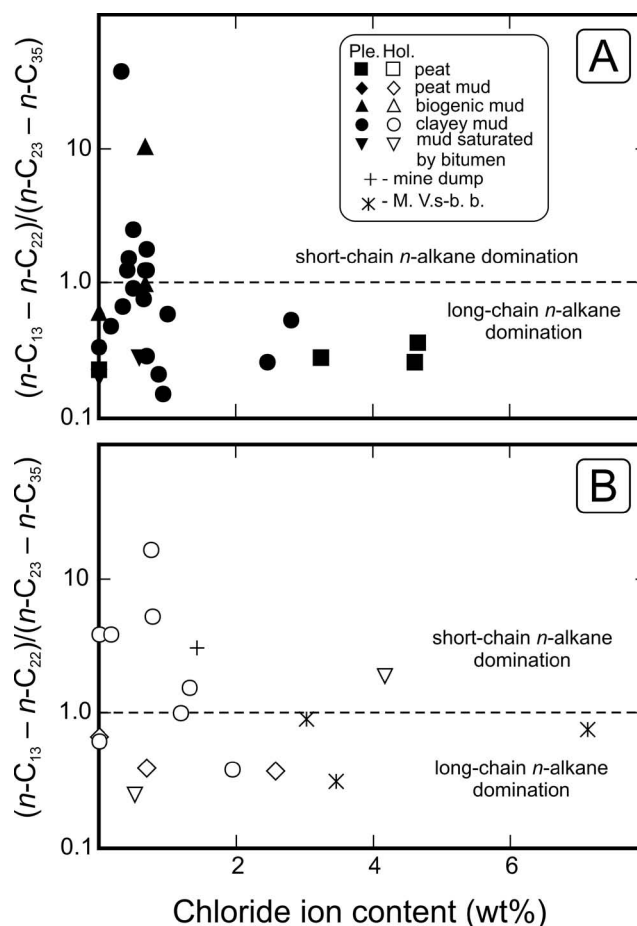
C_{27} dia/C_{27} reg = a ratio of C_{27} diasteranes to C_{27} regular steranes (Mello et al., 1988); Pr/C_{30} hop = Pristane/C_{30}-17,21 (H)-29-hopane TIC;

-- compounds not found.
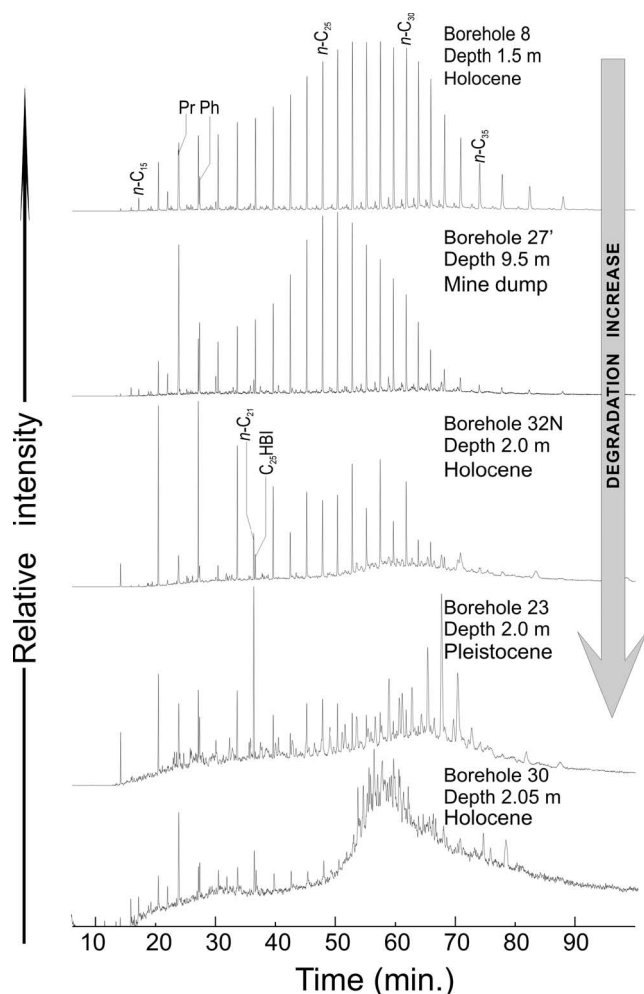
Fig. 3. Examples of *n*-alkane and isoprenoid distributions in bitumen. Pr – pristane; Ph – phytane, HBI – high-branched isoprenoid

Fig. 4. Short-chain (n-C_{13} – n-C_{22})/long-chain (n-C_{23} – n-C_{35}) *n*-alkanes ratio in bitumen versus chloride ion content in (**A**) Pleistocene sediments, and (**B**) Holocene sediments, salt-bearing Miocene Vorotyshcha beds, and mine wastes. Ple. – Pleistocene, Hol. – Holocene, M.V.s-b.b. – Miocene Vorotyshcha salt-bearing beds

mulations are the main sources of compounds present in the extracts. Calculated CPI values of extracted bitumen are comparable with those obtained for oil accumulations and seeps in the Starunia area (Kotarba *et al.*, 2005b). High variability of *n*-alkane distribution in bitumen is probably caused by two factors: biodegradation and/or oxidation/evaporation affecting the organic matter sampled at shallow depths. It caused partial or total removal of *n*-alkanes from some of bitumens (Fig. 3) and, in the case of Nos 30/2.05 and 30/1.4 samples, partly affected sterane distribution (*m/z* = 217) (Fig. 5). The presence of unbiodegraded bitumen in the less consolidated mine dump (Sample 27'/9.5, Fig. 5) most probably is connected with inflow of the fresh oil from the neighbouring rocks. However, pentacyclic triterpane distribution is not altered by the process. 25-Norhopanes (m/z = 177), assumed to be by-products of hopanes' degradation in anaerobic conditions, are absent, indicating rather oxic conditions of biodegradation (Peters *et al.*, 2005).

Comparing three ranges of *n*-alkanes: n-C_{13} – n-C_{18}, n-C_{19} – n-C_{24} and n-C_{25} – n-C_{40} with sediment lithology and age (Fig. 6), we found that Holocene clayey muds (Fig. 6C) and, to some extent, also Pleistocene clayey muds (Fig. 6A) show a clear trend reflecting the gradual removal of

short chain *n*-alkanes. The Miocene samples (Nos 4/5.0, 22/6.6 and 22/7.9) are clustered in the diagram (Fig. 6C) and reveal *n*-alkanes composition comparable to oils from deep accumulations and surface seeps (Kotarba *et al.*, 2005b), which indicates their limited degree of degradation.

The other factor affecting composition of saturate hydrocarbons is an input of even numbered carbon atoms *n*-alkanes, mostly short-chained (n-C_{14} – n-C_{20}) together with brine (see Fig. 3 in sample No. 32N/2.0 and, to lesser extent, also No. 23/2.0). Domination of these compounds over odd numbered carbon atoms *n*-alkanes is an unusual feature. Grimalt and Albaiges (1987) and ten Haven *et al.* (1988) attributed such proportions to organic matter from hyper- saline reducing environments, whereas Elias *et al.* (1997) observed this effect in recent sediments where *n*-alkenes accompany *n*-alkanes. As in our samples *n*-alkenes are absent, we suggest that hydrocarbons might have migrated from deep-seated reservoirs (*e.g.* Kliwa Sandstones within the Menilite Shales) and might have been dissolved in brines accumulated in the salt-bearing Miocene Vorotyshcha beds as well as in Pleistocene and Holocene sediments. The absence of any other compounds related to hypersaline environment (gammacerane, elevated content of pentakishomo-
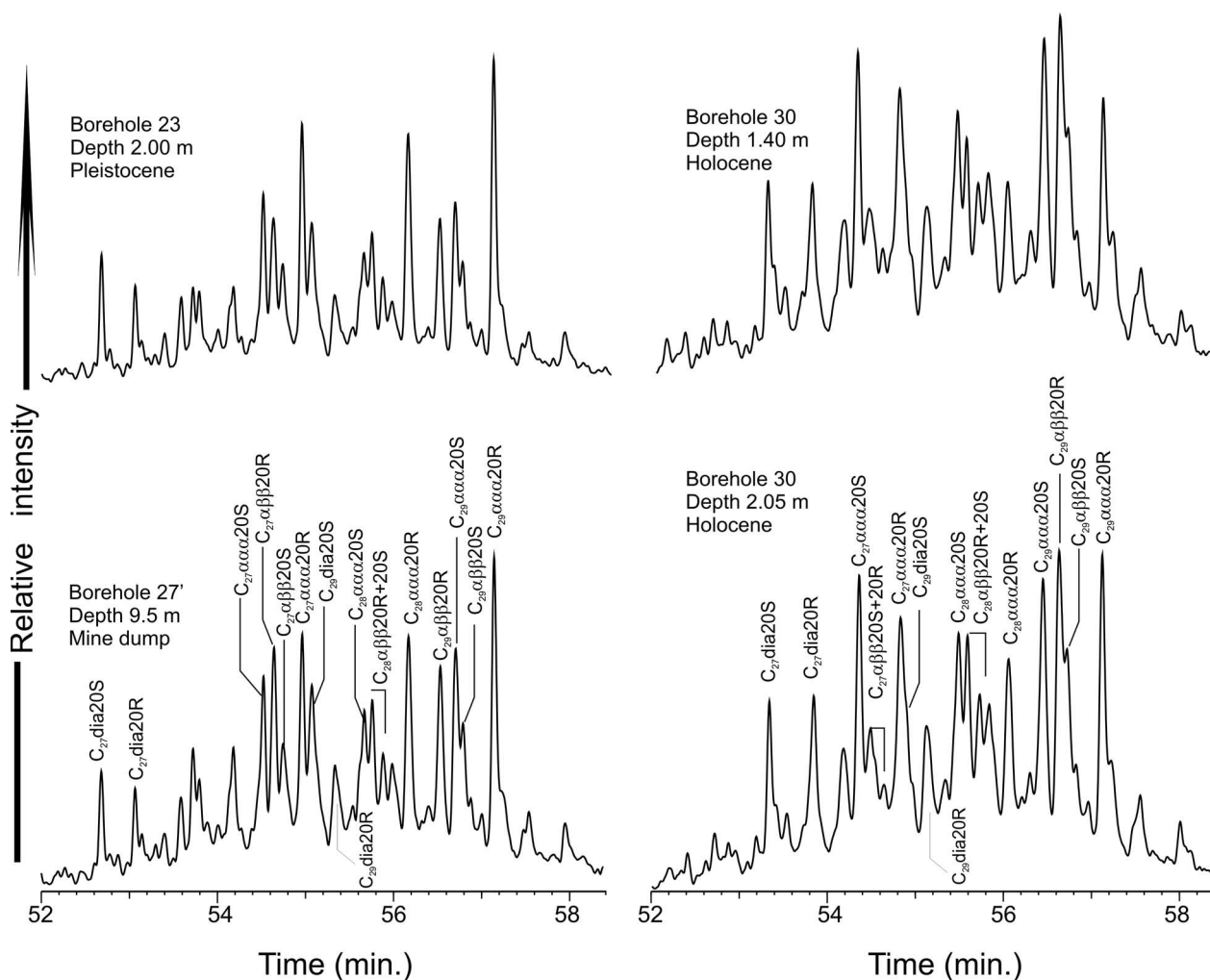
Fig. 5. Examples of steranes distribution in bitumen; typical distribution represented by Pleistocene sample No. 23/2.00, strongly degraded bitumen – by Holocene samples Nos 30/1.40 and 30/2.05, and unbiodegraded bitumen – by mine waste sample No. 27'/9.5; C_{27}dia20S – diacholestane 20S, C_{27}dia20R – diacholestane 20R, $C_{27}\alpha\alpha\alpha20S$ – C_{27}-5α,14α,17α(H)-cholestane 20S, $C_{27}\alpha\beta\beta20S+20R$ – C_{27}-5α,14β,17β(H)-cholestanes 20S and 20R, $C_{27}\alpha\alpha\alpha20R$ – C_{27}-5α,14α,17α(H)-cholestane 20R, C_{29}dia20S – diastigmastane 20S, C_{29}dia20R – diastigmastane 20R, $C_{28}\alpha\alpha\alpha20S$ – C_{28}-5α,14α,17α(H)-ergostane 20S, $C_{28}\alpha\beta\beta20S+20R$ – C_{28}-5α,14β,17β(H)- ergostanes 20S and 20R, $C_{28}\alpha\alpha\alpha20R$ – C_{28}-5α,14α,17α(H)-stigmastane 20R, $C_{28}\alpha\alpha\alpha20S$ – C_{28}-5α,14α,17α(H)-stigmastane20S, $C_{29}\alpha\beta\beta20S$ – C_{29}-5α,14β,17β(H)-stigmastane 20S, $C_{29}\alpha\beta\beta20R$ – C_{29}-5α,14β,17β(H)-stigmastane 20R, $C_{29}\alpha\alpha\alpha20R$ – C_{29}-5α,14α,17α(H)-stigmastane 20R

hopanes) confirms such migration mechanism as short-chain *n*-alkanes show the highest solubility in water among all aliphatic compounds present in mature organic matter. Preferential removal of *n*-alkanes over heavier aliphatic compounds is a well known result of water washing (Palmer, 1993; Jovancicevic *et al.*, 2005). Moreover, brine enhances the solubility of aliphatic compounds in aqueous phase in comparison to freshwater (Peake & Hodgson, 1966; Boehm & Quinn, 1973). As a result, *n*-alkanes are the most probable compounds to be transported in brines, which may enrich the distribution of *n*-alkanes driven from migrating bitumen in short-chain, even numbered carbon atoms *n*-alkanes (see Fig. 3, sample No. 32N/2.0). Concentrations of even numbered carbon atoms *n*-alkanes are much higher than those of odd numbered carbon atoms, particularly in the *n*-C_{14} – *n*-C_{20} range, indicating that biodegradation occurred prior to the enrichment in even numbered carbon atoms *n*-alkanes, since these compounds are not degraded. The greater the extent of earlier degradation, the more brine-derived *n*-alkanes affect the extract composition. The input of even numbered carbon atoms *n*-alkanes also change the CPI values in a few samples, in which CPI values are significantly lower than 1.00 (Nos 1/2.6, 22/4.8, 28/3.4, 30/1.4 and 30/7.2 samples). In Fig. 7, the plot of two *n*-alkane ratios: *n*-C_{16}/*n*-C_{17} (which is the short chain *n*-alkane range mostly affected by brine input) and *n*-C_{22}/*n*-C_{23} (being the end of a short-chain *n*-alkane range, *i.e.*, least-affected by brine input) represents two group of samples: (i) those without large input of brine *n*-alkanes (*i.e.*, where both *n*-C_{16}/*n*-C_{17} and *n*-C_{22}/*n*-C_{23} ratios are about 1.0) and (ii) those with variable input of brine *n*-alkanes causing the increase of *n*-C_{16}/*n*-C_{17} ratio when the input is low or moderate and the increase of both ratios when the input is very high. In rare cases when the input of brine
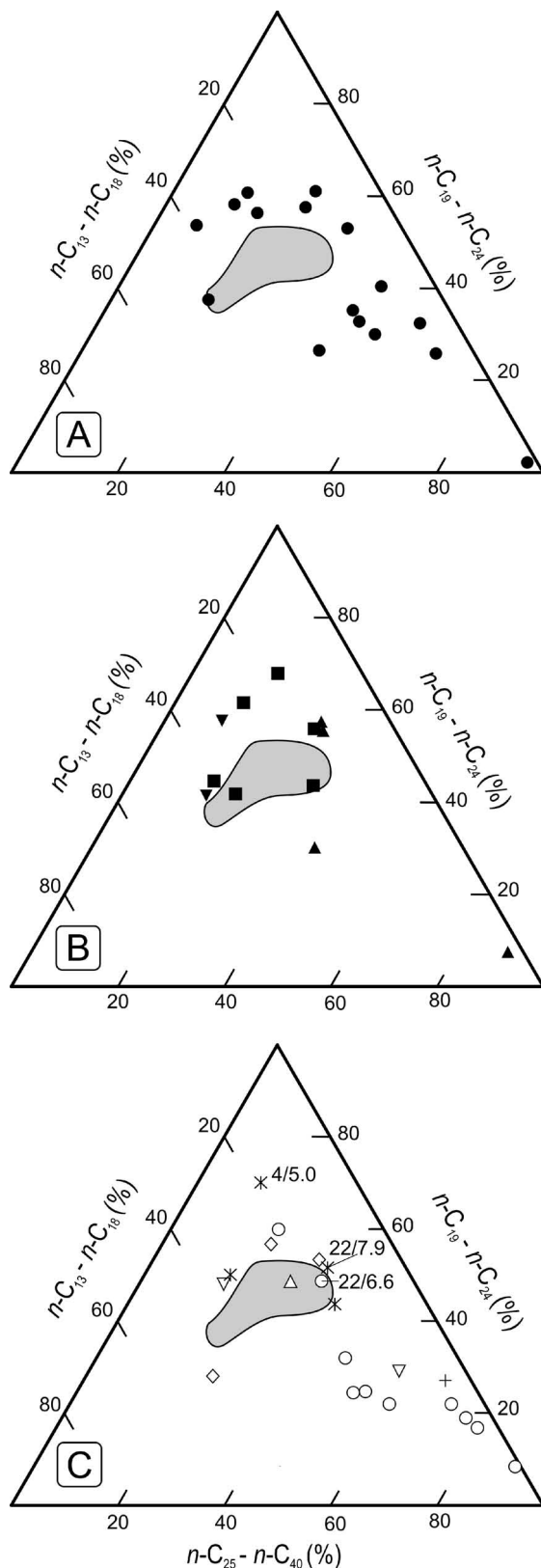
Fig. 6. Ternary diagram of *n*-alkane composition of bitumen extracted from (**A**) Pleistocene clayey muds, (**B**) Pleistocene peat, biogenic muds and muds saturated with bitumen, (**C**) Holocene sediments, salt-bearing Miocene Vorotyshcha beds, and mine wastes. For key to bitumen samples see Table 1. Shaded area represents oils from surface seeps, from skull of the "second" woolly rhinoceros in Starunia, and the nearest deep accumulations, after Kotarba *et al.* (2005b). For explanations of symbols see Fig. 4
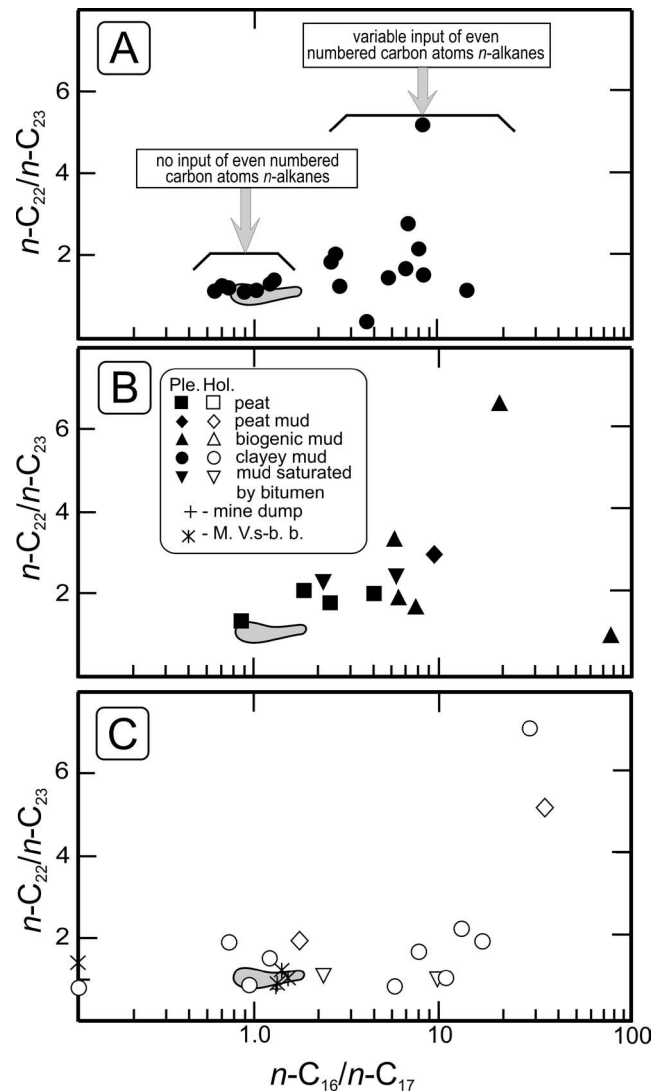
Fig. 7. *n*-C_{22}/*n*-C_{23} versus *n*-C_{16}/*n*-C_{17} ratios for bitumen extracted from (**A**) Pleistocene clayey muds, (**B**) Pleistocene peat, biogenic muds and muds saturated with bitumen, (**C**) Holocene sediments, salt-bearing Miocene Vorotyshcha beds, and mine wastes. Shaded area represents oils from surface seeps, from skull of the "second" woolly rhinoceros in Starunia, and the nearest deep accumulations, after Kotarba *et al.* (2005b)

n-alkanes is very high it enriches extracts in both *n*-C_{16} and *n*-C_{22} increasing values of both ratios. This input is chaotic, showing no correspondence to lithology, borehole locality or depth. Moreover, this effect is most prominent in samples with low bitumen yield and/or with strongly degraded bitumen. In these bitumens, brine-transported *n*-alkanes with even-carbon-atom-number predominance are dominating constituents. As most of Holocene clayey muds have a large input of even numbered carbon atoms *n*-alkanes (Fig. 7C), the influence of increasing degradation on *n*-alkane distribution is well-marked.

The Pr/Ph ratio values (Table 2) range between 1.10 and 2.08 in the majority of samples. However, sample No. 23/2.0 shows exceptionally low Pr/Ph ratio (0.29). Its organic matter is highly degraded (Fig. 3), which probably affects the pristane concentrations. High variability of

Pr/n-C$_{17}$ and Ph/n-C$_{18}$ ratios values (Table 2) results from two degradation processes described above. The changes of these ratios are evident despite lithology or stratigraphy of sampling sites (Fig. 6). The most degraded hydrocarbons occur in Pleistocene clayey mud (samples Nos 30N/8.8, 42/6.45 and 42/8.5), unlike the extracts from Miocene strata (Table 2, Fig. 8C). The latter are characterized by indices values comparable to those of oils known from this area (Kotarba *et al.*, 2005b).

The distribution of pentacyclic triterpanes from 18α(H)-22, 29, 30-trisnorneohopane (Ts) to 17α, 21β(H)-pentakishomohopanes (C$_{35}$) is uniform in all analysed n-hexane extracts (Fig. 9). C$_{30}$-17α, 21β(H)-hopane (abbreviated as C$_{30}$αβ hopane) shows the highest concentration, followed by C$_{29}$αβ norhopane. Moretanes (β,α hopanes) are present in minor quantities, while β,β-hopanes (biological configuration) and hopenes (indicators of immature organic matter) are absent despite the shallow sampling depths. Such pentacyclic triterpane distribution is typical of thermally mature organic matter, corresponding at least to the middle stage of catagenesis ("oil window") (Seifert & Moldowan, 1978). Advanced maturity of the analysed bitumen is also indicated by the presence of C$_{29}$ Ts-18α (H)-30-norneohopane (abbreviation: C$_{29}$Ts) in all analysed extracts. Such hopane distribution is similar to that previously found in crude oils from the study area, with hopane maturity parameters showing comparable values (Kotarba *et al.*, 2005b). The values of 17α(H),21β(H)-29-homohopane geodiastereomers ratio [abbreviated as C$_{31}$αβ 22S/ (22R+22S)] in extracted bitumen have reached the maximum 0.56, typical of "oil window" maturity (Table 2, compare with Tab. 6 in Kotarba *et al.*, 2005b). Both the near-surface bitumen considered in this paper and the oils and extracts from the Menilite Shales (Kotarba *et al.*, 2005b, 2007) contain oleanane (-s). Its presence in migrating crude oil indicates that terrestrial organic matter contained in it (Fig. 9) is Late Cretaceous or younger in age, since this compound originates from β-amyrin – the substance occurring in angiosperms tissues (Ekweozor & Udo, 1988; Moldowan *et al.*, 1994; ten Haven *et al.*, 1993). The oleanane/C$_{30}$ hopane ratio shows slightly larger variability of values in surface samples than other pentacyclic triterpanes parameters and falls into the range from 0.08 (sample No. 32N/2.0) to 0.40 (sample No. 4'/1), with an average of about 0.21 (Table 2). It is possible that the elevated value in No. 4'/1 sample is caused by extensive biodegradation of organic matter, since there are some indicators of slightly higher oleanane resistance to biodegradation compared to hopanes (Wenger *et al.*, 2002). However, such removal order is often reversed, and hopanes considered to be more resistant are removed prior to less resistant steranes or even diasteranes prior to hopanes (Peters *et al.*, 2005). Due to this, it is difficult to propose a satisfactory conclusion. It is also possible that the investigated bitumen was supplied from at least two different sources of crude oil, both of similar maturity but slightly differing in organic geochemical signatures, among them oleanane contents. This concept seems to be confirmed by highly branched isoprenoid (HBI), probably of diatom origin (ten Haven *et al.*, 1993), which is present only in some analysed bitumens while absent in the others (Fig. 3). Since
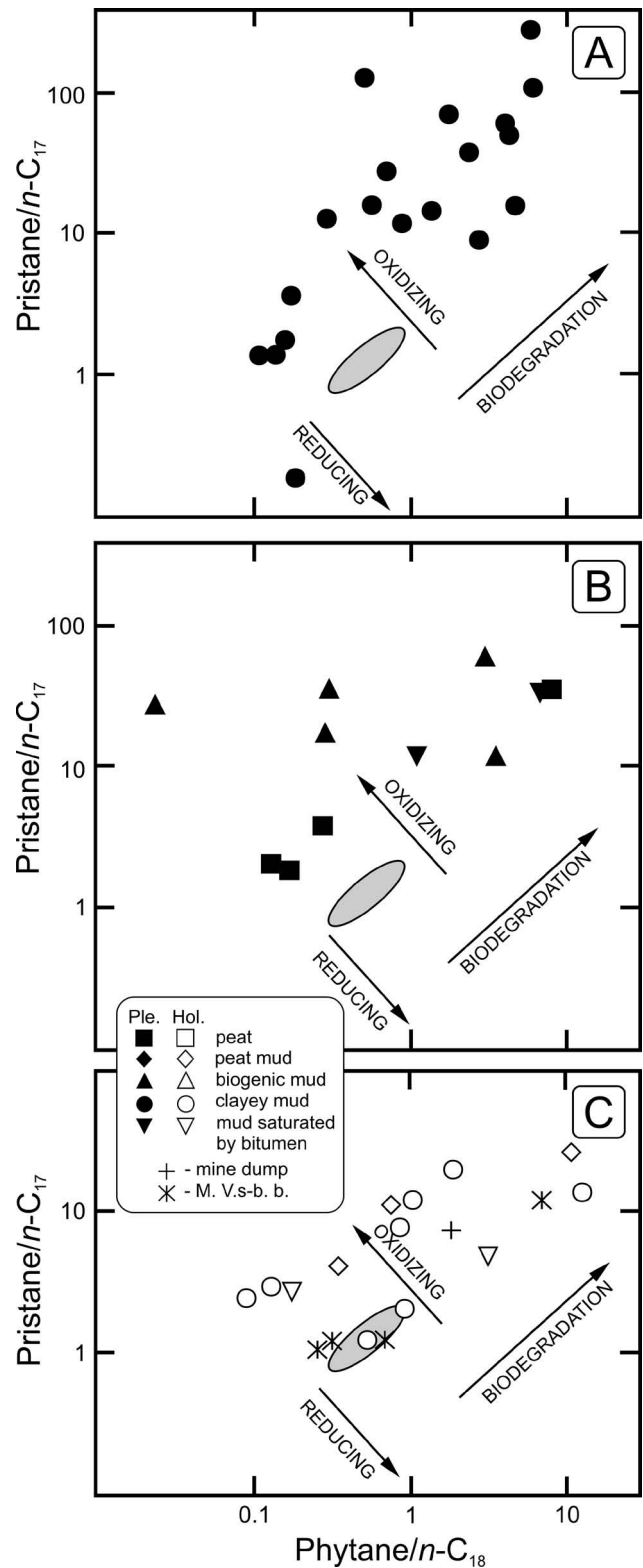
Fig. 8. Pristane/n-C$_{17}$ versus phytane/n-C$_{18}$ ratio identifying secondary processes in bitumen extracted from: (**A**) Pleistocene clayey muds, (**B**) Pleistocene peat, biogenic muds and muds saturated with bitumen, and (**C**) Holocene sediments, salt-bearing Miocene Vorotyshcha beds, and mine wastes. Direction of secondary processes after Shanmugam (1985). Shaded area represents oils from surface seeps, from skull of the "second" woolly rhinoceros in Starunia, and the nearest deep accumulations, after Kotarba *et al.* (2005b). Ple. – Pleistocene, Hol. – Holocene, M.V.s-b.b. – Miocene Vorotyshcha salt-bearing beds
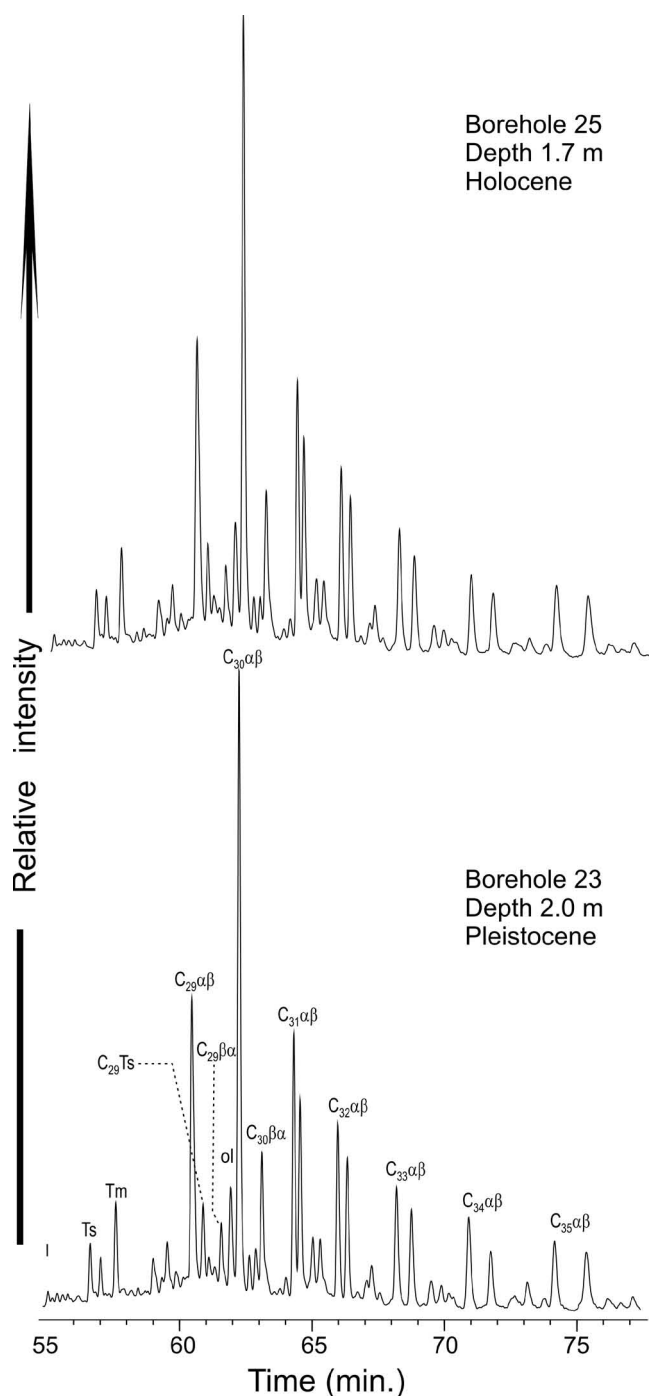
Fig. 9. Example of hopanes distribution in bitumen from No. 25/1.7 Holocene sample (upper part) and No. 23/2.0 Pleistocene sample (lower part). Ts – 18α(H)-22,29,30-trisnorneohopane, Tm – 17α(H)-22,29,30-trisnorhopane, $C_{29}αβ$ – C_{29}-17α,21β(H)-29-norhopane, $C_{29}βα$ – C_{29}-17α,21β(H)-29-norhopane, C_{29}Ts – 18α(H)-30-norneohopane, ol – oleanane, $C_{30}αβ$ – C_{30}-17α, 21β(H)-29-hopane, $C_{30}βα$ – C_{30}-17β,21α(H)-29-hopane, $C_{31}αβ$ – C_{31}-17α,21β(H)-29-homohopanes, $C_{32}αβ$ – C_{32}-17α,21β(H)-29-bishomohopanes, $C_{33}αβ$ – C_{33}-17α,21β(H)-29-trishomohopanes, $C_{34}αβ$ – C_{34}-17α,21β(H)-29-tetrakishomohopanes, $C_{35}αβ$ – C_{35}-17α,21β(H)-29-pentakishomohopanes
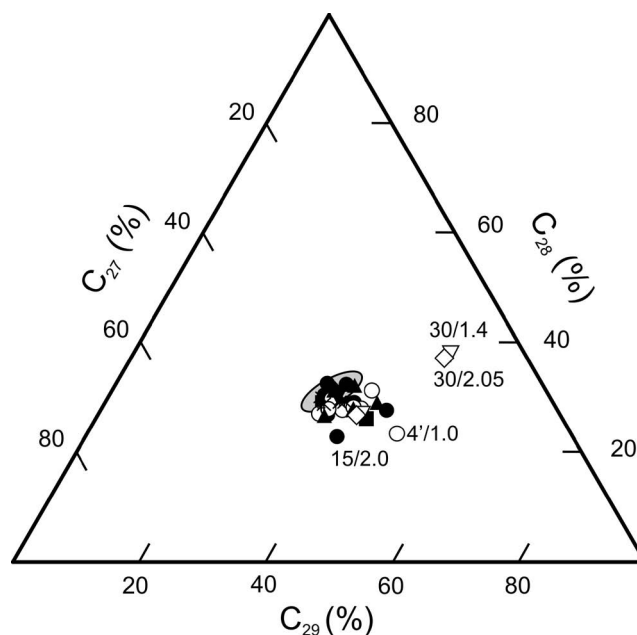
Fig. 10. Ternary diagram of sterane composition in bitumen. Shaded area represents oils from surface seeps, from skull of the "second" woolly rhinoceros in Starunia, and the nearest deep accumulations, after Kotarba *et al.* (2005b). For key to samples see Table 2, for explanation of symbols see Fig. 8

only a few previously analysed crude oils from the Starunia area (Kotarba *et al.*, 2005b) contained the HBI, it is possible that such variability in its occurrence in surface bitumen is caused by contribution from at least two sources: one containing the HBI and one devoid of it.

In a majority of analysed samples sterane distributions are uniform with almost equal amounts of cholestane (C_{27}), ergostane (C_{28}) and stigmastane (C_{29}) diastereomers present in the extracts (Fig. 10, Table 2). Diasteranes formed from sterols in the presence of clay minerals occur in all extracts. Also, the ratios of total C_{29} regular steranes versus total C_{27} regular steranes (C_{29}/C_{27} ster. in Table 2) are similar, falling into the range from 0.90 to 1.77, except for highly biodegraded No. 30/1.4 and 30/2.05 samples, which show increased contents of stigmastane diastereomers due to removal of cholestanes (C_{29}/C_{27} ratios are 3.47 and 4.41, respectively). The Huang-Meinschein ternary diagram (Fig. 10) shows sub-equal relative concentrations of cholestane, ergostane and stigmastane diastereomers (Huang-Meinschein, 1979), which roughly correspond to those found in previously analysed crude oils from the Starunia area (Kotarba *et al.*, 2005b). Sterane maturity parameters shown in Table 2 indicate the same stage of thermal evolution in all samples: values of the ratio of C_{29}-5α,14α,17α(H) (S+R)-stigmastane 20S to its total diastereomers 20S and 20R vary around 0.34, whilst the ratios of C_{29}-5α,14β,17β(H)-stigmastanes to its total diastereomers are about 0.35 in a majority of samples. However, both sterane parameters are much more influenced by biodegradation than hopane parameters and exhibit higher variability. There are two samples showing significantly elevated values of both parameters: Nos 30/1.4 and 30/2.05 (0.60 and 0.46, 0.54 and 0.46,
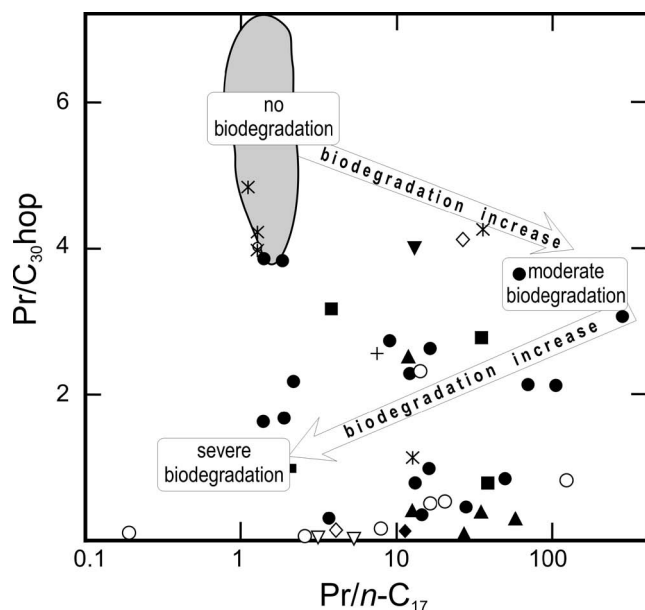
Fig. 11. Pristane/C_{30} hopane versus pristane/n-C_{17} ratios indicating biodegradation range of bitumen. Shaded area represents oils from surface seeps, from skull of the "second" woolly rhinoceros in Starunia, and the nearest deep accumulations, after Kotarba *et al.* (2005b). For explanation of symbols see Fig. 8

Table 3

Assessment of biodegradation stage of the investigated bitumen from the Starunia area according to the biodegradation scale of Connan (1984)

| Bio-degradation stage	Changes in composition caused by biodegradation	Samples
0	not degraded	8/1.5, 28/9.8
1	slightly degraded, lighter *n*-alkanes affected	15/0.2, 15/2.0, 22/2.5, 22/3.5, 22/4.0, 22/4.8, 22/5.5, 22/6.6, 22/7.9, 27/9.5, 28/6.0, 30/7.2, 30N/4.6, 31/4.0, 32N/5.0, 42/3.8, 42/4.6
2	slightly degraded, most *n*-alkanes removed	1/2.6, 4/4.15, 4'/4.0, 7/10.4, 15/4.0, 15/6.0, 22/1.0, 23/2.0, 23/4.0, 25/1.7, 25/2.6, 30N/8.2, 30N/8.8, 28/3.4, 28/4.7, 30/6.8, 30N/2.9, 30N/5.0, 30N/7.2, 30N/7.3, 38/12.9, 42/6.45, 42/8.4, 42/8.5
3	moderately degraded, no *n*-alkanes; Pr and Ph removed or their distribution affected	5/6.5, 4/3.5, 4/4.0, 4.50, 4'/1.0, 4'/3.7, 4'/4.85, 30/4.6, 30/5.8, 30/6.8, 32N/2.0
5 - 6	strongly degraded, sterane distribution affected	30/1.4, 30/2.05

Pr – pristane; Ph – phytane

respectively). Because in both samples sterane distribution was affected by degradation (see Fig. 10), such change may be caused by this process. However, this may result also from an input of more mature bitumen mixing with main dominating bitumen, showing higher values of these two parameters. The latter assumption is confirmed by elevated values of C_{29}Ts to C_{30}-α,β-hopane ratio (but not by the other hopane parameters) found for these two samples. As geological environment of the Starunia area was highly modified by mining operations, such an input might have occurred in the sediments sampled at a particular depth, while it might have been absent from other sediments sampled in the same borehole. Because these two samples show very large input of brine-transported *n*-alkanes (CPI values 0.60 and 0.90, Table 2), it is likely that their sterane distribution has been enriched in the same way. Moreover, this bitumen contains a much wider range of *n*-alkanes, up to *n*-C_{30}, which makes input of heavier compounds from brines more probable.

The extent of biodegradation processes in extracted bitumen is variable. Using the biodegradation scale of Connan (1984), it was possible to assess their alteration in analysed samples (Table 3).

Pentacyclic triterpanes were unaffected by biodegradation and a norhopane series indicative of anoxic conditions of the process has not been found in the extracts (Peters *et al.*, 2005). The extent of biodegradation is shown in a plot of Pr/C_{30} hop versus Pr/n-C_{17} (Fig. 11). The initial removal of *n*-alkanes caused the increase in Pr/n-C_{17} ratio values (0–2 stages of biodegradation, Table 3). Moderately degraded samples show very high values of this ratio, even up to 277 (sample No. 42/8.5; Table 2). The next stages (3–6) are marked by a gradual decrease of both ratios as a

result of pristane removal and increase of relative concentration of C_{30}-17α21β(H)-29-hopane.

The intensity of biodegradation process depends on chloride ion content. In samples where high chloride ion contents were determined the Pr/n-C_{17} ratio is low. Samples characterized by chloride ion contents below 1 wt% usually show high Pr/n-C_{17} ratios (Fig. 12) indicating the initial stages of biodegradation process. Other observed population of samples, characterized by low chloride ion contents and Pr/n-C_{17} ratios, include mainly Pleistocene and Holocene clayey muds and peat/biogenic muds, *i.e.*, materials which underwent severe biodegradation.

It was found that biodegradation of the analysed organic matter is variable and unrelated to the sampling depth (Table 3). Shallower samples are often less degraded than the deeper ones, such as in No. 4' borehole, where samples Nos 4'/3.7 and 4'/4.85 have lost all *n*-alkanes while samples Nos 4'/1.0 and 4'/4.0 have retained them. Similar but less obvious relationships were found in Nos 30N and 30 boreholes, where bitumen is highly biodegraded. Fig. 11 shows examples of depth distribution of biodegradation-dependent Pr/n-C_{17} and Pr/C_{30} hop ratios in selected boreholes. The Miocene strata (boreholes Nos 28 and 22) are less degraded. These are characterized by low Pr/n-C_{17} values (below 1.5) and high Pr/C_{30} hop ones (over 3.9) (Table 2, Fig. 13). The range of biodegradation of bitumen in Quaternary sediments is variable and independent of depth and stratigraphy, as described above.

The oil collected from the "upper oil eye" (Kotarba *et al.*, 2005b) localized near the borehole No. 15 is not biode-
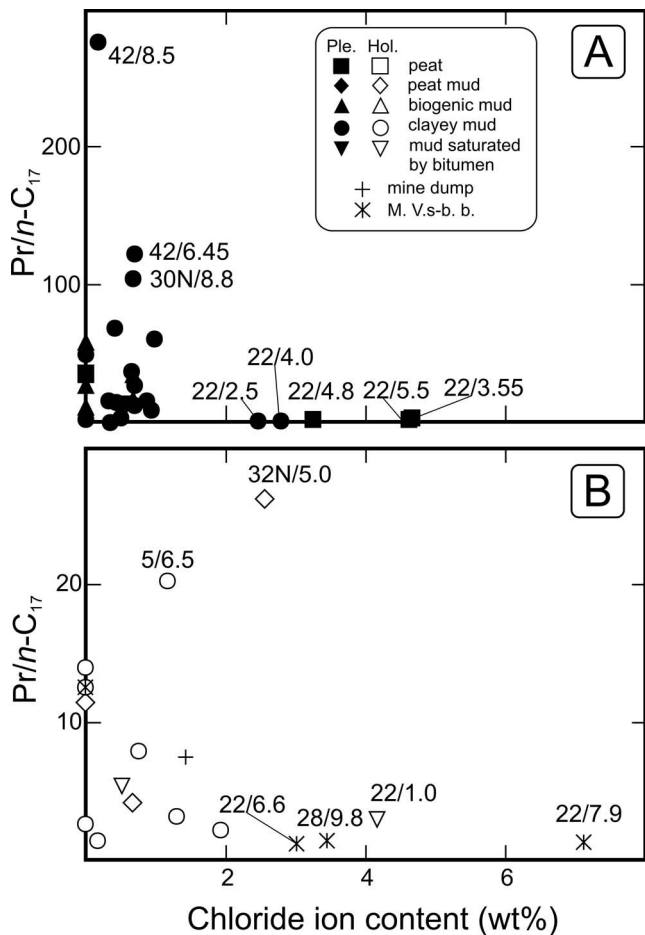
Fig. 12. Pristane/*n*-C$_{17}$ ratio of extracted bitumen versus chloride ion content in (**A**) Pleistocene sediments and (**B**) Holocene sediments, salt-bearing Miocene Vorotyshcha beds, and mine wastes. Ple. – Pleistocene, Hol. – Holocene, M.V.s-b.b. – Miocene Vorotyshcha salt-bearing beds

graded, unlike bitumen extracted from the near-surface sediments. Probably, the oil pool is a result of leakage from old Kaufman-1 well (Fig. 1), whose casing terminates at shallow depth below the ground level. The same situation is observed when comparing oil indices from the "lower oil eye" (Kotarba *et al.*, 2005b) and bitumen form the neighbouring boreholes Nos 1 and 25 (Fig. 1). Hydrocarbons from these boreholes are severely biodegraded (Table 2), while the oil parameters are comparable to those of deeply accumulated oils. This oil probably originates from leaking casing in the Juliusz-2 well (Fig. 1).

Taking into consideration the biomarker indices, the most favourable conditions for preservation of the remains of extinct, giant vertebrates occur in the vicinity of boreholes Nos 22 and 23, where the statistically highest Pr/C$_{30}$ hop and the lowest Pr/*n*-C$_{17}$ ratios were observed (Table 2). These data indicate also that the least favourable preservation conditions are in the vicinity of boreholes Nos 1, 4, 4'
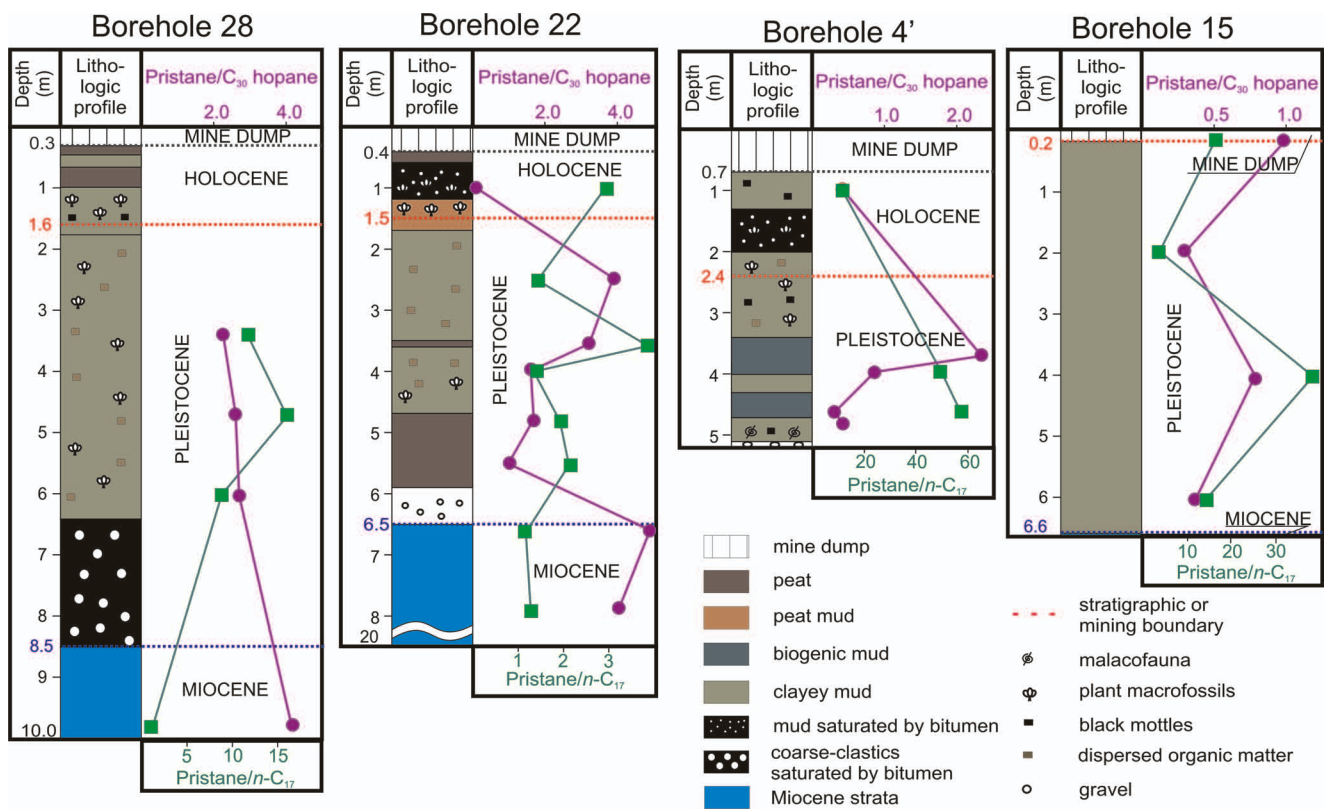
Fig. 13. Depth distribution of biomarker indices in lithological columns of selected boreholes Nos 4', 15, 22 and 28. For location of boreholes see Fig. 1, for explanations of formulae see Table 2
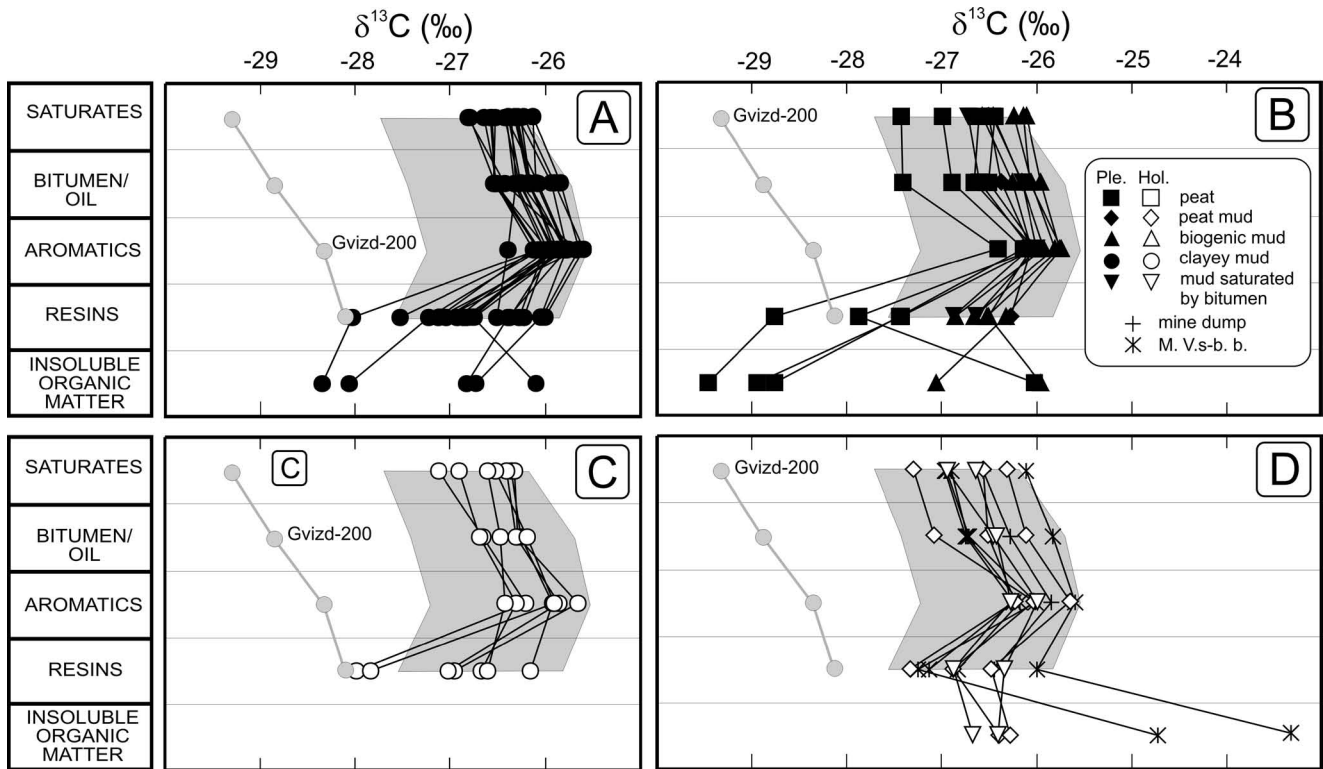
Fig. 14. Isotopic curves of bitumen from (**A**) Pleistocene clayey muds, (**B**) Pleistocene peat, biogenic muds and muds saturated with bitumen, (**C**) Holocene clayey muds, and (**D**) other Holocene sediments, salt-bearing Miocene Vorotyshcha beds, and mine wastes. Shaded area represents oils from surface seeps, from skull of the "second" woolly rhinoceros in Starunia, and the nearest deep accumulations, after Kotarba *et al.* (2005b). Ple. – Pleistocene, Hol. – Holocene, M.V.s-b.b. – salt-bearing Miocene Vorotyshcha beds

and 15. The important indicator of extensive changes in organic matter, preventing the preservation of mammal remnants, is the inconsistency of biodegradation. Its rapid and chaotic changes from depth to depth without any general trend may indicate rocks strongly disturbed by mining operations. Probably, the shafts enabled the invasion of oxygen and freshwater into sediments, which might have destroyed the fossils.

Stable carbon isotope analysis

Stable carbon isotope compositions of extracted bitumens and their fractions vary in a narrow range: saturated hydrocarbons from –27.4 to –26.1‰ (difference 1.3‰), aromatics from –26.4 to –25.6‰ (difference 0.8‰), resins from –28.8 to –26.0‰ (difference 2.8‰) and bitumen from –27.4 to –25.8‰ (difference 1.6‰) (Fig. 14). This suggests that all analysed bitumens originate from the common source, regardless of the reservoir. Comparing these results with the stable carbon isotope composition of oils from deep accumulations and from surface seeps (Kotarba *et al.*, 2005b), the isotopic homogeneity of analysed samples is evident (Figs 14, 15). Because the influence of biodegradation on stable carbon isotopes is weaker than on biomarkers (Peters *et al.*, 2005), these parameters enabled us to correlate even the strongly degraded samples. Insoluble organic matter present in the studied sediments has a wide range of stable carbon isotope compositions: from –29.5 to –23.3‰ (difference 6.2‰) (Fig. 14), which indicates diversified sources.

CONCLUSIONS

The results of geochemical studies of biomarker distribution and stable carbon isotopes of bitumen extracted from the Pleistocene and Holocene sediments in the Starunia area indicate that:

– all bitumens originate from oil-prone, Type II kerogen with insignificant admixture of terrestrial Type III kerogen. Their biomarkers and isotopic compositions correspond to crude oils occurring in deep accumulations in the vicinity of Starunia and suggest that these oils were the sole source of bitumens found in the near-surface sediments. The stable carbon isotope composition reveals that the bitumen is not genetically connected with residual organic matter present in the studied Quaternary sediments;

– the thermal maturity of all investigated bitumens is very similar and can be estimated as middle stage of catagenesis. Some input of other, more mature bitumen cannot be excluded. Input of immature organic matter to bitumen from Pleistocene and Holocene sediments has not been found, because no indicators such as *n*-alkenes, hopenes or sterenes were detected in the analysed extracts. The possible reason of this are very low concentrations of saturated and aromatic hydrocarbons in very immature organic matter of the Pleistocene and Holocene sediments compared to the large input of these compounds from mature crude oils migrating from the nearest deep accumulations of the Boryslav-Pokuttya and Skyba units of the Carpathians;

– the main factors differentiating the near-surface bitu-
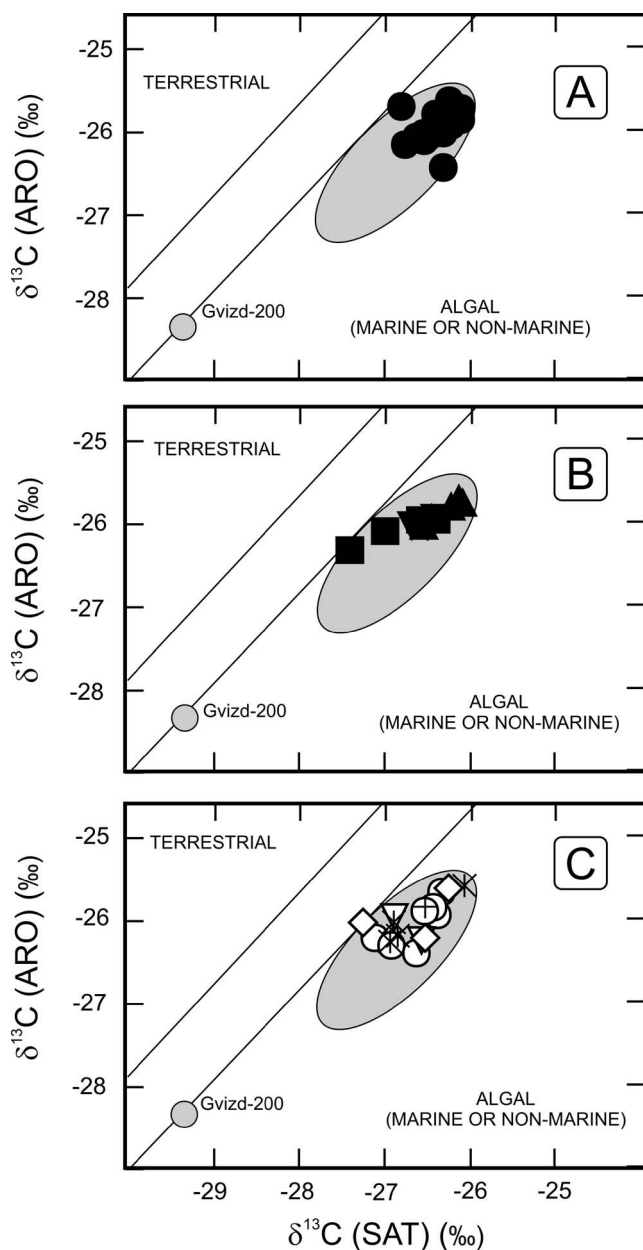
Fig. 15. Stable carbon isotope composition of aromatic hydro-carbons versus saturated hydrocarbons for bitumen extracted from (**A**) Pleistocene clayey muds, (**B**) Pleistocene peat, biogenic muds and muds saturated with bitumen, and (**C**) Holocene sediments, salt-bearing Miocene Vorotyshcha beds, and mine wastes. Shaded area represents oils from surface seeps, from skull of the "second" woolly rhinoceros in Starunia, and the nearest deep accumulations, after Kotarba *et al.* (2005b). Genetic fields after Sofer (1984). For explanation of symbols see Fig. 14

men were: biodegradation, water washing and/or weathering (oxidation/evaporation), which resulted in partial or total removal of *n*-alkanes (as compounds mostly susceptible to such processes) and, in some samples, also acyclic isoprenoids and steranes. Biodegradation degree varies from 0 to 6;

– additional evidence of brine-transported bitumen was found, particularly in previously biodegraded and/or weath-

ered organic matter. The main compounds transported in this way were even numbered carbon atoms *n*-alkanes: *n*-tetradecane, *n*-hexadecane and *n*-octadecane. Transport in solution precluded the presence of less water-soluble compounds, such as most of biomarkers – indicators of hypersaline environment;

– the most favourable conditions for preservation of large, extinct mammals within the Pleistocene muds exist in the vicinity of Nos 22 and 23 boreholes, where bitumen is best preserved. The worst conditions were found in the vicinity of Nos 1, 4, 4' and 15 boreholes;

– intensification of biodegradation and weathering effects followed by irregular scattering, and chaotic changes of bitumen properties caused by mining operations seem to indicate the unfavourable conditions for preservation of the remnants of Pleistocene mammals.

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